Synthesis and Characterization of Environment-Sensitive Fluorescent Ligands for Human 5-HT$_{1A}$ Receptors with 1-Arylpiperazine Structure[†]

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A series of "long-chain" 1-(2-methoxyphenyl)piperazine derivatives containing an environment-sensitive fluorescent moiety (4-amino-1,8-naphthalimide, 4-dimethylaminophthalimide, dansyl) was synthesized. The compounds displayed very high to moderate 5-HT$_{1A}$ receptor affinity and good fluorescence properties. 6-Amino-2-[5-[4-(2-methoxyphenyl)-1-piperazinyl]pentyl]-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-dione (**4**) combined very high 5-HT$_{1A}$ receptor affinity (K_i = 0.67 nM), high fluorescence emission in CHCl$_3$, and undetectable fluorescence emission in aqueous solution. It was evaluated for its ability to visualize 5-HT$_{1A}$ receptors overexpressed in CHO cells by fluorescence microscopy.

Introduction

The serotonin1A (5-HT$_{1A}$) receptor is an important member of the large family of serotonin receptors and is perhaps the most extensively studied for several reasons. One is the availability of the selective agonist 8-OH-DPAT[a], which has allowed extensive biochemical, physiological, and pharmacological characterization of the receptor. The 5-HT$_{1A}$ receptor was the first serotonin receptor to be cloned and sequenced. Human, rat, and mouse 5-HT$_{1A}$ receptors have been cloned and their amino acid sequences deduced. More importantly, the receptor has been stably expressed in a number of neural and non-neural cell lines.[1] It is generally accepted that 5-HT$_{1A}$ receptor is involved in anxiety and depression. Recently, it has been suggested that 5-HT$_{1A}$ receptor agonists have neuroprotective properties, whereas 5-HT$_{1A}$ receptor antagonists could be useful in the treatment of cognitive disorders related to Alzheimer's disease.[2] Transfection of the cDNA of this receptor into cells has resulted in the acquisition of large amounts of information regarding signal transduction pathways linked to the receptor (adenylyl cyclase inhibition, coupling to phospholipase, regulation of ion channels) and pharmacological properties of the receptor. The realization that the 5-HT$_{1A}$ receptor couples to multiple signaling pathways in cells and tissues in which it is normally expressed reflects the promiscuity of GPCR signaling pathways and the fact that single receptor subtypes might be linked to various second messengers in a single cell system.[3] It is likely that the pharmacology of GPCRs differs markedly depending on their membrane localization and the signaling proteins present wi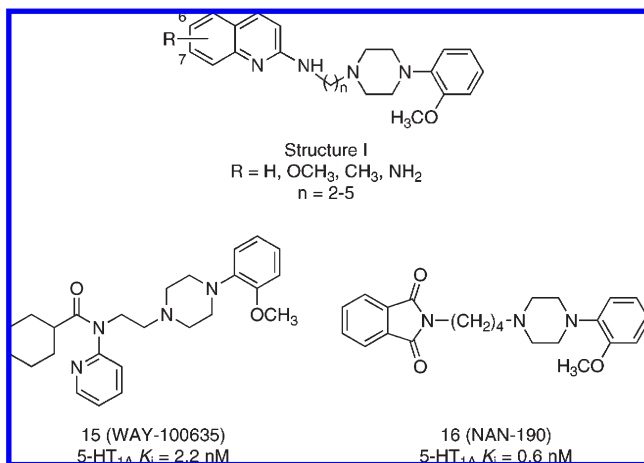thin that locality. This might have important implications for the orchestration of drug action within a given cell type and the potential for cross-talk with other signaling pathways.[4] The study of receptor pharmacology at single cell level may benefit from use of fluorescent ligands. They offer a multiplicity of information such as the mechanism of ligand binding, the movement and internalization of receptors in living cells, the distances between ligands and fluorescently labeled amino acids, the physical nature of the binding pocket, and the visualization of labeled receptors.[5]

Thus, the identification of fluorescent ligands for 5-HT$_{1A}$ receptor would be of interest. Fluorescent ligands are usually designed starting from the selection of a pharmacophoric moiety that is conjugated with a fluorescent tag through an appropriate linker. For GPCRs activated by biogenic amines, the ligand binding sites are buried within the transmembrane regions of the receptor and interact with small molecules. Consequently, appropriate positioning of the fluorophore within the final conjugate is critical to retain both receptor binding affinity and efficacy. Thus, this structural modification could significantly affect the pharmacological properties of the parent ligands.[6] As an alternative strategy, we have recently designed fluorescent probes for 5-HT$_{1A}$ receptor by incorporating the fluorophore moieties into the pharmacophoric part of the ligand. In other words, the fluorescent moiety itself had structural characteristics that could be well-tolerated in the interaction with the target receptor. In this way, a series of fluorescent ligands was generated, with the possibility of optimizing the fluorescence properties and receptor affinity at the same time. In particular, we described a series of fluorescent *N*-[ω-[4-(2-methoxyphenyl)-1-piperazinyl]alkyl]-2-quinolinamines (Chart 1, structure I) characterized by nanomolar affinity at 5-HT$_{1A}$ receptor, along with acceptable fluorescence properties.[7] However, the ligands showed excitation wavelengths in the near UV region of the spectrum (340−380 nm) and this could limit their use to visualizing 5-HT$_{1A}$ receptors overexpressed in cell lines by conventional confocal laser scanning microscopy due to cell

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[a] Abbreviations: 8-OH-DPAT, 8-hydroxy-*N*,*N*-dipropylaminotetraline; GPCR, G-protein coupled receptor.

Chart 1. Structures of 5-HT$_{1A}$ Receptor Agents

Structure I
R = H, OCH$_3$, CH$_3$, NH$_2$
n = 2-5

15 (WAY-100635)
5-HT$_{1A}$ K_i = 2.2 nM

16 (NAN-190)
5-HT$_{1A}$ K_i = 0.6 nM

Scheme 1[a]

13a: n = 2
13b: n = 3
13c: n = 4
13d: n = 5

14a-d

[a] Reagents: (A) hydrazine hydrate, 10% Pd/C.

autofluorescence. To overcome this, we have replaced the 2-quinolinamine with three fluorescent labels possessing different fluorescent properties. The fluorophores were 4-amino-1,8-naphthalimide (6-amino-1,3-dioxo-1*H*,3*H*-benzo-[de]isoquinolinyl), 4-dimethylaminophthalimide (5-dimethylamino-1*H*-isoindole-1,3(2*H*)-dione), and dansyl (5-dimethylaminonaphthalene-1-sulfonyl), which are known to have different excitation wavelengths (315 nm for the dansyl moiety, and 450−470 nm for the others). Moreover they possess the additional feature of being environment-sensitive. These fluorophores exhibit a low quantum yield in aqueous solution but become highly fluorescent in nonpolar solvents or when bound to a hydrophobic site in proteins or membranes. Environment-sensitive molecules may be useful in fluorescence microscopy studies, where fluorescence will be higher for ligand−receptor interaction and lower for the unbound ligand in aqueous environment.

Here we report the design, synthesis, and binding properties for the human 5-HT$_{1A}$ receptor of the new ligands **1−12**. The fluorescent properties of the ligands were determined in CHCl$_3$, PBS buffer solution, and in the presence of cell membranes overexpressing the receptor. We also report some results of visualization of fluorescent ligand-labeled cells using fluorescence microscopy.

Chemistry. The synthesis of the final compounds **1−12** (Scheme 1) required the key amines **13a−d**, which were prepared according to the literature as detailed in Supporting Information (SI). For target compounds **1−4**, key amines **13a−d** were condensed with 4-nitro-1,8-naphthalic anhydride to give nitro derivatives **14a−d**, which were subsequently reduced by catalytic hydrogenation with hydrazine hydrate in the presence of 10% Pd/C. The other target compounds were prepared from the condensation of **13a−d** with 4-(dimethylamino)phthalic acid in the presence of 1,1′-carbonyldiimidazole (**5−8**) or with dansyl chloride (**9−12**).

Results and Discussion

The affinity values for 5-HT$_{1A}$ receptor and fluorescent properties of the target compounds **1−12** are displayed in Table 1.

To develop the new series of fluorescent probes for 5-HT$_{1A}$ receptors, we took advantage of existing structure−activity relationship studies on 5-HT$_{1A}$ receptor ligands with *N*1-substituted *N*4-arylpiperazines (the "long-chain"
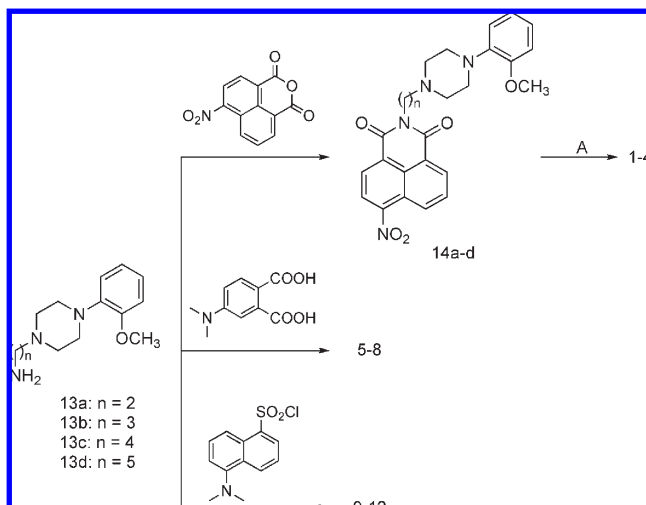
arylpiperazines). The general formula of these compounds presents a 1-arylpiperazine linked through an alkyl chain to a terminal fragment, belonging to one of the following four structural classes: (i) imides, (ii) amides, (iii) alkyl, arylalkyl, or heteroarylalkyl derivatives, and (iv) tetralins.[8] In particular, we focused our study on 1-(2-methoxyphenyl)piperazine derivatives because they frequently display high affinity for the 5-HT$_{1A}$ receptor as for the antagonists **15** (WAY-100635)[9] and **16** (NAN-190)[10] (Chart 1). As outlined above, several examples from the literature indicate that various terminal fragments are well-tolerated. Thus, the replacement of 2-quinolinamine nucleus in structure I (Chart 1) with the fluorescent moieties 4-amino-1,8-naphthalimide, 4-dimethylaminophthalimide, and dansyl should be tolerated with respect to the 5-HT$_{1A}$ affinity. In doing this, we were encouraged by the structural similarity between the terminal fragment of the 5-HT$_{1A}$ antagonist **16** and the 4-dimethylaminophthalimide fluorophore. Finally, optimization of the intermediate alkyl chain length was accomplished because it is well-documented that each combination arylpiperazine/terminal fragment requires a spacer of appropriate length.[8] Overall, our initial considerations were confirmed by K_i values of the target compounds, which ranged between 0.47 and 151 nM. In particular, considering the 4-amino-1,8-naphthalimide derivatives **1−4**, we note that length of the spacer greatly influence the affinity. The compound **4** with a five methylene spacer displayed subnanomolar affinity (K_i = 0.67 nM). When the linker was shortened, a considerable loss in affinity was observed (> 65-fold), especially for compounds **1** (n = 2) and **2** (n = 3), whereas **3** was only 10-fold less potent than **4**. A different trend was found in the group of 4-dimethylaminophthalimide derivatives **5−8**. The compounds **6** (n = 3) and **7** (n = 4) displayed subnanomolar affinity (K_i = 0.47 and 0.50 nM, respectively), whereas **5** (n = 2) and **8** (n = 5) were 30- and 15-fold less potent. Compound **7** is formally derived from **16** by introduction of a dimethylamino substituent in the 4-position of the phthalimide moiety. This modification had no influence on affinity: in fact, both compounds displayed K_i values in the nanomolar range (0.50 and 0.60 nM, respectively).[10] The use of dansyl as terminal fragment was also tolerated, although none of the derivatives showed subnanomolar affinity at 5-HT$_{1A}$ receptor. K_i values of compounds **9−12** were within a narrow range

(29−151 nM). It is likely that the different spatial orientation of dansyl group with respect to that of phthalimido or naphthalimido is less favorable for interaction with the 5-HT$_{1A}$ receptor. Overall, affinity data showed that various terminal fragments are well-tolerated with respect to the 5-HT$_{1A}$ receptor affinity and that the optimization of the intermediate alkyl chain length is a key step when studying "long-chain" arylpiperazines. Considering the fluorescent properties in ethanol of the target compounds **1−12**, we note that the structural modification of the fluorescent core has little or no influence on either excitation or emission wavelengths.[11,12] The excitation wavelengths of the dansyl derivatives **9−12** are more shifted toward the UV region of the spectrum as compared to the 4-dimethylaminophthalimide derivatives **5−8** (400−420 nm) and the 4-amino-1,8-naphthalimides **1−4** (440−441 nm). All the target compounds showed high difference of excitation to emission maximal wavelengths (Stokes shift). To probe the environment affecting the sensitivity of the fluorescent ligand, the quantum yield (Φ) in PBS buffer and apolar solvent CHCl$_3$ were determined (Table 1). **1−12** exhibited very low fluorescence in PBS buffer but became fluorescent in CHCl$_3$. In particular, compounds **1**, **5**, and **9**, characterized by a two methylene spacer, showed the lower Φ value within each series. Compounds **2**, **4**, and **7** displayed the highest Φ values in CHCl$_3$ (Φ = 0.67, 0.59, 0.86, respectively). Taken together, 5-HT$_{1A}$ receptor affinity data and fluorescence properties showed that the pursued optimization strategy was successful, especially for derivatives **4** and **7**, which combined very high 5-HT$_{1A}$ receptor affinity (K_i = 0.67 and 0.50 nM, respectively), high Φ values in CHCl$_3$, and excitation and emission wavelengths in a suitable range for visualization experiments by confocal laser scanning microscopy. On this basis, compounds **4** and **7** were incubated with

cell membranes overexpressing human 5-HT$_{1A}$ receptor to evaluate how the interaction of the fluorescent ligand with the
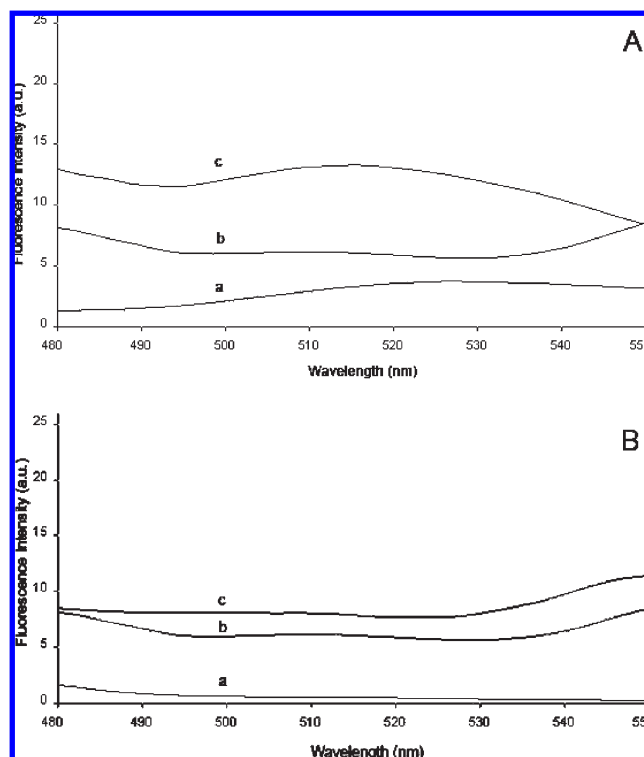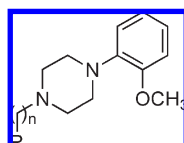

Figure 1. Spectrofluorimetric determination of the fluorescence of **4** (A) or **7** (B) in the presence of cell membranes overexpressing 5-HT$_{1A}$ receptors (c) in comparison with cell autofluorescence (b) and PBS buffer alone (a).

Table 1. 5-HT$_{1A}$ Receptor Affinities and Fluorescence Properties of the Final Compounds **1−12**[a]

comp	R	n	K_i, nM ± S.E.M.	EtOH λ_{exc} (nm)	EtOH λ_{em} (nm)	CHCl$_3$ λ_{exc} (nm)	CHCl$_3$ λ_{em} (nm)	Φ (PBS)	Φ (CHCl$_3$)
1		2	44 ± 4	441	520	406	500	∼ 0	0.20
2		3	116 ± 7	440	520	403	497	∼ 0	0.67
3		4	6.8 ± 0.5	440	520	403	495	0.035	0.53
4		5	0.67 ± 0.11	440	520	400	496	0.012	0.59
5		2	15 ± 1.4	400	512	395	481	∼ 0	0.10
6		3	0.47 ± 0.50	400	512	395	488	0.010	0.50
7		4	0.50 ± 0.01	406	520	397	490	∼ 0	0.86
8		5	7.8 ± 0.4	420	520	395	482	∼ 0	0.43
9		2	73 ± 4	337	508	342	482	∼ 0	0.34
10		3	29 ± 1	337	508	341	480	0.018	0.36
11		4	151 ± 12	335	507	340	482	0.020	0.36
12		5	54 ± 7	335	507	344	482	0.020	0.42

[a] The values are the mean ± SEM from three independent experiments in triplicate.

receptor reflects on the fluorescence emission. Figure 1 shows typical fluorescence curves representing the fluorescence emission of the ligands in PBS, the autofluorescence of cell membrane alone, and the total observed fluorescence due to the membranes incubated with **4** and **7**, respectively. The incubation of the fluorescent ligands with cell membranes led to a marked increase in fluorescence emission intensity when compared to PBS solution for both compounds. The naphthalimide derivative **4** determined a 3-fold increase of fluorescence emission when compared to cell membrane autofluorescence, whereas **7** determined only a slight increase in fluorescence emission. Therefore, compound **4** was selected for labeling 5-HT$_{1A}$ receptor in CHO cells. The experiments were performed by conventional confocal laser scanning microscopy at a λ_{exc} = 458 nm for single photon excitation and at 900 nm for multiphoton excitation. In the first attempt to visualize 5-HT$_{1A}$ receptors, stably transfected CHO cells (TCHO) were incubated with **4** at different concentrations (0.125, 0.5, and 1 μM) at ~23 °C. In parallel, the same experiments were performed on wild-type CHO cells to evaluate if the binding of the fluorescent ligand was specific. Compound **4** exhibited reasonably bright fluorescence when imaged under these experimental conditions. Representative fluorescence micrographs under various conditions are shown in Figure 2. Unfortunately, **4** appeared to undergo almost instantaneous nonspecific aggregation as well as internaliza-
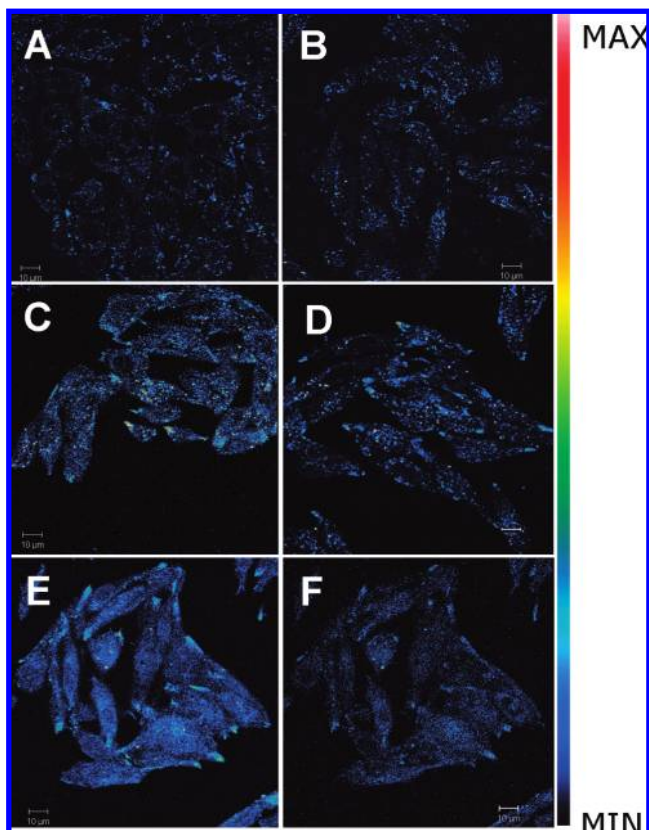

Figure 2. Representative fluorescent micrographs (color coded for intensity scale) of CHO and TCHO cells labeled with **4** upon single and multiphoton excitation: (A) CHO and (B) TCHO cells labeled with 0.125 μM of **4** at 4 °C and excited at 458 nm (single photon excitation), TCHO cells labeled with (C) 0.5 and (D) 1 μM of **4** at ~23 °C and excited at 900 nm (multiphoton excitation), (E) TCHO cells labeled with 0.125 μM of **4** at ~23 °C and excited at 458 nm, and (F) the same field of cells (as in (E)) excited at 900 nm. The scale bar represents 10 μm in all cases.

tion upon addition to cells. The patterns of labeling exhibited are similar in both TCHO and CHO cells, suggesting predominantly nonspecific interactions. To inhibit endocytosis, labeling was also performed at 4 °C without appreciable improvement in the labeling pattern. Moreover, competition with 5-HT did not induce any perceivable change in the labeling pattern. Therefore, **4** appeared to be unsuitable for visualizing 5-HT$_{1A}$ receptors specifically in CHO cells. An explanation for the observed high nonspecific binding is difficult to address. It has been proposed that high lipophilicity is a factor contributing to the nonspecific binding of some fluorescent probes.[13-15] However, literature data do not suggest an optimal lipophilicity range that might be associated with low nonspecific binding for fluorescent ligands. Also, specific interaction of compound **4** with molecular targets other than 5-HT$_{1A}$ receptors present in CHO cells cannot be ruled out. For instance, it has been reported that 1,8-naphthalimide derivatives can strongly interact with DNA.[16] Clearly, these possibilities cannot be properly evaluated during the design process.

Conclusions

We have identified a series of environment-sensitive fluorescent ligands for 5-HT$_{1A}$ receptors by inserting an environment-sensitive moiety in the *N*-[4-(2-methoxyphenyl)-1-piperazinyl]alkyl framework. Several of the newly prepared ligands displayed nanomolar affinity at 5-HT$_{1A}$ receptor and fluorescent properties suitable for use in fluorescence microscopy. In particular, the fluorescent ligand **4** showed a favorable combination of 5-HT$_{1A}$ receptor affinity (K_i = 0.67 nM), Stokes shift (excitation wavelength = 400 nm, emission wavelength = 496 nm), and quantum yield in CHCl$_3$ (Φ = 0.59). In spite its favorable profile, compound **4** was unable to specifically label the human 5-HT$_{1A}$ receptor overexpressed in CHO cell lines.

Experimental Section

General Procedure for Preparation of Nitro Derivatives 14a−d. A mixture of 4-nitro-1,8-naphthalic anhydride (0.48 g, 2.0 mmol) and the appropriate amine **13a−d** (2.2 mmol) in absolute EtOH (20 mL) was refluxed for 3 h. After cooling, the solvent was evaporated to dryness and the crude residue was chromatographed (CHCl$_3$/AcOEt, 1:1, as eluent) to afford pure compound **14a−d** as a brown semisolid.

2-[5-[4-(2-Methoxyphenyl)-1-piperazinyl]pentyl]-6-nitro-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-dione (14d). Yield 40%. ^{1}H NMR (CDCl$_3$) δ 1.41−1.51 (m, 2H), 1.63−1.72 (m, 2H), 1.76−1.85 (m, 2H), 2.47 (t, 2H, *J* = 7.4 Hz), 2.69 (br s, 4H), 3.11 (br s, 4H), 3.83 (s, 3H), 4.25 (t, 2H, *J* = 7.4 Hz), 6.83−7.02 (m, 4H), 7.99 (t, 1H, *J* = 8.2 Hz), 8.42 (d, 1H, *J* = 8.0 Hz), 8.69 (d, 1H, *J* = 8.0 Hz), 8.75 (dd, 1H, *J* = 0.8, 7.4 Hz), 8.84 (dd, 1H, *J* = 0.8, 8.7 Hz). ESI$^+$/MS *m/z* 503.0 (MH$^+$). ESI$^+$/MS/MS *m/z* 340 (72), 297 (82), 255 (100).

General Procedure for Preparation of Final Compounds 1−4. Hydrazine hydrate (0.15 mL, 3 mmol) was added to a solution of nitro derivatives **14a−d** (1.0 mmol) in absolute EtOH (20 mL). 10% Pd/C (50 mg) was added portionwise to the warm solution, and the mixture was refluxed for 1 h. After cooling, the catalyst was removed by filtration through celite and the solvent was evaporated to dryness. H$_2$O (20 mL) was added to the residue, and the aqueous phase was extracted with CHCl$_3$ (2 × 30 mL). The collected organic layers were dried over Na$_2$SO$_4$ and evaporated under reduced pressure. The crude residue was chromatographed (CHCl$_3$/MeOH, 19:1, as eluent) to afford pure compound **1−4** as yellow solid.

6-Amino-2-[5-[4-(2-methoxyphenyl)-1-piperazinyl]pentyl]-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-dione (4). Yield 40%. ^{1}H NMR (CDCl$_3$) δ 1.41−1.51 (m, 2H), 1.59−1.69 (m, 2H), 1.71−1.81 (m, 2H), 2.48 (app t, 2H), 2.69 (br s, 4H), 3.11 (br s, 4H), 3.85 (s, 3H), 4.16 (t, 2H, J = 7.4 Hz), 5.01 (br s, 2H, D$_2$O exchanged), 6.83−7.02 (m, 5H), 7.64 (d, 1H, J = 7.5 Hz), 8.11 (d, 1H, J = 8.5 Hz), 8.40 (d, 1H, J = 8.0 Hz), 8.58 (d, 1H, J = 7.4 Hz). ESI$^+$/MS m/z 473.0 (MH$^+$). ESI$^+$/MS/MS m/z 281 (28), 225 (100); mp 210−212 °C (from CHCl$_3$/*n*-hexane). Anal. (C$_{28}$H$_{32}$N$_4$O$_3$) C, H, N.

Fluorescence Spectroscopy. Emission and excitation spectra of compounds **1**−**12** were recorded as detailed in SI. Fluorescence quantum yields were calculated in reference to that of quinine sulfate in 0.5 M H$_2$SO$_4$ as a standard (excitation wavelength 350 nm; Φ = 0.546),[17] as detailed in SI.

Fluorescent Labeling of Cells. Fluorescence microscopy observation in CHO cells overexpressing human 5-HT$_{1A}$ receptors were performed as detailed in SI.

Supporting Information Available: Spectral data of compounds **1**−**3**, **5**−**12**, and **14a**−**c**. Biological methods and statistical analysis. Experimental procedure for fluorescence spectroscopy. Fluorescent labeling of HEK-293 cell membranes expressing the h5-HT$_{1A}$ receptor. Fluorescent labeling of CHO cells expressing the h5-HT$_{1A}$ receptor. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Pucadyil, T. J.; Kalipatnapu, S.; Chattopadhyay, A. The serotonin$_{1A}$ receptor: a representative member of the serotonin receptor family. *Cell. Mol. Neurobiol.* **2005**, *25*, 553–580.

(2) Lacivita, E.; Leopoldo, M.; Berardi, F.; Perrone, R. 5-HT$_{1A}$ receptor, an old target for new therapeutic agents. *Curr. Top. Med. Chem.* **2008**, *8*, 1024–1034.

(3) Raymond, J. R.; Mukhin, Y. V.; Gettys, T. W.; Garnovskaya, M. N. The recombinant 5-HT$_{1A}$ receptor: G protein coupling and signalling pathways. *Br. J. Pharmacol.* **1999**, *127*, 1751–1764.

(4) Cordeaux, Y.; Hill, S. J. Mechanisms of cross-talk between G-protein coupled receptors. *Neurosignals* **2002**, *11*, 45–57.

(5) Daly, C. J.; McGrath, J. C. Fluorescent ligands, antibodies, and proteins for the study of receptors. *Pharmacol. Ther.* **2003**, *100*, 101–118.

(6) Leopoldo, M.; Lacivita, E.; Berardi, F.; Perrone, R. Developments in fluorescent probes for receptor research. *Drug Discovery Today* **2009**, *14*, 706–712.

(7) Lacivita, E.; Leopoldo, M. *N*-[ω-[4-(2-Methoxyphenyl)-1-piperazinyl]alkyl]-2-quinolinamines as high-affinity fluorescent 5-HT$_{1A}$ receptor ligands. *J. Med. Chem.* **2008**, *51*, 1492–1495.

(8) Lopez-Rodriguez, M. L.; Ayala, D.; Benhamu, D.; Morcillo, M. J.; Viso, A. Arylpiperazine derivatives acting at 5-HT$_{1A}$ receptors. *Curr. Med. Chem.* **2002**, *9*, 443–469.

(9) Chemel, B. R.; Roth, B. L.; Armbruster, B.; Watts, V. J.; Nichols, D. E. WAY-100635 is a potent dopamine D$_4$ receptor agonist. *Psychopharmacology* (*Berlin*) **2006**, *188*, 244–251.

(10) Raghupathi, R. K.; Rydelek-Fitzgerald, L.; Teitler, M.; Glennon, R. A. Analogues of the 5-HT$_{1A}$ serotonin antagonist 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine with reduced alpha 1-adrenergic affinity. *J. Med. Chem.* **1991**, *34*, 2633–2638.

(11) Saroja, G.; Soujanya, T.; Ramachandram, B.; Samanta, A. 4-Aminophthalimide derivatives as environment-sensitive probes. *J. Fluoresc.* **1998**, *8*, 405–410.

(12) Grabchev, I.; Chovelon, J.-M.; Qian, X. A copolymer of 4-*N*,*N*-dimethylaminoethylene-*N*-allyl-1,8-naphthalimide with methylmethacrylate as a selective fluorescent chemosensor in homogeneous systems for metal cations. *J. Photochem. Photobiol. A,* **2003**, *158*, 37–43.

(13) Koman, A.; Kolb, V. M.; Terenius, L. Prolonged receptor blockade by opioid receptor probes. *Pharm. Res.* **1986**, *3*, 56–60.

(14) Korlipara, V.; Ells, J.; Wang, J.; Tam, R.; Elde, S.; Portoghese, P. Fluorescent *N*-benzylnatrindole analogues as potential delta opioid receptor selective probes. *Eur. J. Med. Chem.* **1997**, *32*, 171–174.

(15) Kshirsagar, T.; Nakano, A. H.; Law, P.-Y.; Elde, R.; Portoghese, P. S. NTI4F: a non-peptide fluorescent probe selective for functional delta opioid receptors. *Neurosci. Lett.* **1998**, *249*, 83–86.

(16) Filosa, R.; Peduto, A.; Micco, S. D.; Caprariis, P.; Festa, M.; Petrella, A.; Capranico, G.; Bifulco, G. Molecular modelling studies, synthesis and biological activity of a series of novel bisnaphthalimides and their development as new DNA topoisomerase II inhibitors. *Bioorg. Med. Chem.* **2009**, *17*, 13–24.

(17) Meech, S. R.; Phillips, D. Photophysics of some common fluorescence standards. *J. Photochem.* **1983**, *23*, 193–217.